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(54) Title: VACCINE PREPARATIONS

(57) Abstract

Vaccine preparations in stable particulate form are disclosed. An immediate-release preparation comprises an immunogen adsorbed to an aluminium salt adjuvant. A controlled- or delayed-release preparation comprises microspherical particles comprising a continuous matrix of biodegradable polymer containing discrete, immunogen-containing regions.

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VACCINE PREPARATIONS

Field of the Invention

This invention relates to vaccine preparations, and in one particular embodiment it relates to vaccine preparations of the type which are variously described as controlled- or delayed-release vaccines, pulsatile or pulsed-release vaccines and single-shot vaccines. The preparations of the present invention are relevant for use as human and veterinary vaccines, and are provided in the form of a dry powder, which can be subsequently incorporated into a liquid suspension or in a solid pellet or implant for administration. Typically, administration of the vaccine preparation of the present invention in the form of a liquid suspension is by parenteral administration, for example by subcutaneous or intramuscular injection.

Background to the Invention

Delivery of a full course of vaccine in a single dose has held attraction in both human and veterinary medicine and a number of patents and other publications (e.g. U.K. Patent No. 1,567,503) have addressed this possibility. For veterinary applications, the advantages include:

(i) reduced time - animals need be handled only once,

(ii) reduced cost - single veterinary visit and reduced handling costs,

(iii) guaranteed compliance with recommended dose schedule (number of doses, time interval between doses).

In human medicine, the above three advantages are also important with compliance being extremely important in developing countries where repeated access to infants is often not possible. In addition, the pain and suffering associated with vaccination, especially of infants, is an additional reason to favour a single-dose vaccine in human medicine.

Early studies of vaccination using inactivated vaccines (generally tetanus or diphtheria toxoids), have demonstrated the importance of two or more discrete doses of vaccine with an interval of at least 4 weeks, and preferably longer, between doses. A third dose is sometimes necessary to induce an adequate immune response, especially in young animals or infants where transfer of maternal antibodies could interfere with the preliminary immune response.

Recent studies in theoretical immunology have supported these findings and introduced the phrase "affinity maturation". Affinity maturation describes the process whereby plasma cells secreting high affinity antibodies to the desired immunogen are preferentially selected whilst plasma cells secreting antibody of lower affinity are lost. The process involves competition between follicular dendritic cells and plasma cells for antigen binding and thus can only occur effectively in the presence of limiting amounts of antigen. The process of affinity maturation may not commence until 2 to 3 weeks after a primary vaccine dose and it is important that the second dose of antigen not be given until the process is effectively complete. This is readily achievable in a multidose vaccination schedule provided the first dose does not contain too much antigen. However, for this process to be achieved in a single dose delayed-release vaccine, it is important that the second and subsequent doses do not release their antigen payload prematurely. To achieve this, the antigen must be contained within a matrix which has a defined time of degradation. This matrix should be biodegradable, although biocompatible matrixes have been proposed as acceptable. A number of options have been reviewed by Cox & Coulter, 1992.

The major effort to develop delayed release vaccines has centred round the studies of Eldridge *et al.*, 1990; 1991, who used the biodegradable copolymer - polylactide coglycolide to produce antigen-containing microspheres and observed a delayed-release of the antigen contents *in vivo* (see also Australian Patent Specifications Nos. 79929/87 and 33433/89). Similar observations have been reported by Kreuter, 1990 using nanoparticles produced from acrylate polymers. Although the above workers were able to show that the concept of delayed-release vaccines was possible, the process they used in the preparation of the vaccines

suffered from a number of deficiencies making it unsuitable for the routine manufacture of a vaccine. The major problems were:

(i) exposure of biological materials to denaturing chemical and physical conditions, and

(ii) difficulty of scale-up.

(iii) low efficiency of incorporation of hydrophilic compounds (e.g. proteins).

In European patent publication No. 0486959, in the name of Vectorpharma International SpA, there are disclosed controlled release, particulate pharmaceutical compositions containing pharmacologically active substances, the compositions comprising a biodegradable polymer such as polylactic acid, polyglycolic acid and copolymers thereof and/or other polymers including a polysaccharide gellifying and/or bioadhesive polymer, an amphiphilic polymer, an agent modifying the interface properties of the particles and the pharmacologically active substance. In the preparation of the pharmaceutical composition, the polymeric substituents are co-solubilised with the agent modifying the interface properties either in the absence of any solvent or in the minimum necessary amount of solvent, and the pharmacologically active substances then dissolved or dispersed in the polymer solution prior to formation of the final particles, for example by emulsion, extrusion, spray drying or spray congealing techniques. As previously described, this technique suffers from a major disadvantage in that the pharmacologically active substance is directly exposed to the mixture of polymeric compounds together with any solvents therein, which results in the denaturing of biological materials used as the pharmacologically active substance.

It is a principal object of the present invention to provide a vaccine preparation and method for the production thereof wherein the immunogenic material is not exposed to an organic solvent or other organic phase when in soluble form, so as to ensure that there are no conformational changes in the immunogen, in other words to maintain the native structure of the immunogen.

Summary of the Invention.

In accordance with a first aspect of the present invention, there is provided an immediate-release vaccine preparation in stable particulate form, which comprises immunogen adsorbed to an aluminium salt adjuvant.

In accordance with this aspect of the invention, there is also provided a method for the production of an immediate-release vaccine preparation in stable, particulate form as described above, which comprises the steps of forming an aqueous suspension of aluminium salt-adsorbed immunogen and spray-drying said suspension.

Freeze-drying or lyophilisation of similar preparations has been described by Csizer *et al.* (US Pat 4578270). This process has a number of shortcomings, most importantly the need to add large amounts of both dextran and protein so that partial retention of the aluminium gel structure can be achieved (40 and 6-4 mg/ml respectively). This large addition of protein can act to displace vaccine antigens from the aluminium gel and in addition would, in most cases, be immunogenic and as a result tend to swamp the immune response to the vaccine antigen. Other problems associated with lyophilisation are that it is less amenable to large-scale production, equipment costs are significantly higher and the resultant product tends to form flakes rather than free-flowing microgranules.

Surprisingly, the gel-forming nature of aluminium gels is completely retained during spray-drying even in the absence of any other materials (apart from minimal quantities of vaccine antigen, typically 1 to 10 μg/ml) which could exert a stabilising effect. Addition of water to the spray-dried powder results in the instant formation of a typical gel, with sedimentation properties similar to the starting material.

In accordance with a second aspect of the present invention, there is provided a controlled or delayed-release vaccine preparation in stable particulate form, said

particles being microspherical particles comprising a continuous matrix of biodegradable polymer containing discrete, immunogen-containing regions.

In this aspect, the invention also provides a method for the production of a controlled- or delayed-release vaccine preparation in stable, particulate form as described above, which comprises the steps of forming an emulsion of an aqueous suspension comprising the immunogen and optionally an adjuvant in a continuous organic phase having said biodegradable polymer dissolved therein, and subsequently spray-drying the water-in-oil emulsion to form said microspherical particles which comprise a continuous matrix of polymer containing discrete, immunogen-containing regions.

In an alternative method, these microspherical particles are produced by spray-drying a suspension of a particulate immunogen-containing material, preferably an immediate-release vaccine preparation in stable particulate form as broadly described above, and optionally an adjuvant in a continuous organic phase having said biodegradable polymer dissolved therein, to form said microspherical particles comprising a continuous matrix of polymer containing discrete, immunogen-containing regions.

These two processes confer major advantages over methods described previously, e.g. Eldridge *et al.* 1991, O'Hagan *et al.* 1991, Singh *et al.* 1991 and Bodmeier & Cheng 1988. In the processes of Eldridge *et al.* 1991 and Bodmeier & Chen 1988, proteins are directly exposed to the organic solvents required to dissolve the PLG. As a result, antigens are denatured and, because most antigens are water-soluble, poor efficiencies of incorporation result. O'Hagan *et al.* 1991 and Singh *et al.* 1991 devised complex processes to try to overcome these deficiencies. Neither process was amenable to commercial scale, and in addition the former showed poor efficiency of incorporation whilst the latter necessitated injection of large quantities of foreign proteins.

Finally, none of these methods is inherently suited to the simultaneous incorporation of adjuvant.

Both the intermediate-release vaccine preparation of this invention and the controlled- or delayed release vaccine preparation are in the form of microspherical particles, preferably in the range of 10 nm to 250 μm, more preferably in the range of 1 μm to 100 μm.

The vaccine preparations in stable particulate form may be made up into vaccine compositions for administration by combining at least one immediate-release vaccine preparation and/or at least one controlled- or delayed-release vaccine preparation with a carrier or diluent acceptable for pharmaceutical or veterinary use. Suitable carriers or diluents for use in the preparation of vaccine compositions for parenteral administration are well known in the art. Alternatively, the vaccine composition may be produced in the form of a solid pellet or implant with known carrier materials.

Detailed Description of the Invention.

In accordance with the present invention, immunogen-containing microspheres of the controlled- or delayed-release vaccine preparation are produced by a one-step process of manufacture with the potential for a very high throughput. The end-product is a free-flowing powder. As a normal though not essential component of the process, adjuvant is incorporated into these microspheres in association with the immunogen, and this confers a number of advantages:

(i) the immunogen is held in a selected configuration during the drying process,

(ii) adjuvant is available to stimulate the immune system at every pulsed release,

(iii) during *in vivo* residence time, whilst delayed-release polymer is undergoing biodegradation, the immunogen is protected from thermal and perhaps enzymic denaturation by attachment to a solid support.

In work leading to the present invention, it has surprisingly been found that an immediate release composition can be provided in stable, solid dry form since it

has been generally believed that aluminium salt-adsorbed immunogens could not be prepared in powder or other dry form without recourse to complex technology and excessive and unacceptable use of stabilisers (e.g. Csizer *et al.*). In accordance with the first aspect of the present invention, however, it has been found that a stable, solid product can be produced as a free-flowing powder by drying an aluminium salt-adsorbed immunogen produced in aqueous suspension. The immunogen may for example be adsorbed on an aluminium salt adjuvant such as aluminium hydroxide or aluminium phosphate. Preferably, the suspension also contains a protein stabiliser, and suitable stabilisers include, for example, sugars and sugar derivatives such as trehalose, lactose, dextrose and glucosamine. The resultant suspension is then dried, preferably spray-dried, to form a free flowing powder. As previously described it has been found that drying of such an aluminium salt-adsorbed immunogen does not denature the immunogen, nor does it degrade the aluminium salt adjuvant, and in fact results from preliminary experiments show that the immunogenicity of the immunogen may be enhanced in such a powder formulation.

In accordance with the second embodiment of the invention, there is provided a process for the manufacture of controlled- or delayed-release microencapsulated vaccines. This process involves the emulsification of vaccine immunogen, preferably in association with adjuvant, all of which comprises the aqueous phase, into a continuous organic phase in which the biodegradable polymer is dissolved. This water-in-oil emulsion is then spray-dried under suitable conditions such as to generate microspheres which comprise a continuous matrix of the polymer surrounding at least one, but preferably many, pockets of immunogen in association with adjuvant.

It will be noted that in accordance with this process, the emulsion which is formed prior to spray drying is a water-in-oil emulsion, in contrast to the oil-in-water emulsions which are produced in the preparation of the delayed-release vaccine compositions of the prior art mentioned above.

In a modification of the process just described, the microspheres may be produced by spray-drying microdroplets which comprise a suspension of micro-particulate immunogen in a solution of the polymer in organic solvent, the micro-particulate antigen being in a form which does not dissolve in the polymer solution, and preferably being the immediate-release vaccine preparation in stable particulate form described herein.

The vaccine preparations of the present invention are applicable for use with a wide variety of immunogens known in both human and veterinary vaccines, including for example tetanus toxoid, diphtheria toxoid, pertussis extract vaccine, influenza virus, and the like.

The biodegradable polymer used in the present invention may be any polymer substance which is capable of existing in a nonaqueous phase, which is biocompatible and which is capable of delayed breakdown *in vivo*. Suitable polymers include, for example polyesters, polyorthoesters, polyanhydrides and cyanoacrylates, as well as various natural polymers including some proteins and polysaccharides. Particularly suitable polymers for use in accordance with the present invention include homopolymers of D-, L- and DL-polylactic acids (D-PLA; L-PLA; DL-PLA) and polyglycolic acid (PGA), and various copolymers (PLG) thereof. Preferably, in the formation of the water-in-oil emulsion, one or more emulsifiers are used, and suitable emulsifiers include, for example, Tween 80, Span 85 and various lecithins and lecithin-derivatives.

Suitable adjuvants for incorporation into a delayed-release vaccine preparation in accordance with this invention include not only the aluminium salt adjuvants previously described (aluminium hydroxide or aluminium phosphate), but also other particulate and non-particulate adjuvants which are well known in the vaccine field. Suitable adjuvants are described, by way of example, by Cox and Coulter, 1992.

Further features of the vaccine preparations of the present invention and the processes for the preparation thereof will be apparent from the following non-limiting Examples.

EXAMPLE 1

Preparation of an Immediate-Release Tetanus Vaccine.

Clostridium tetani was cultured in a protein-free casein hydrolysate medium for 6 days, at which time approximately 60 Lf/ml (*in vitro* flocculation units) of tetanus toxin had been produced. Bacterial cells and debris were removed by centrifugation then the toxin concentrated and washed on a 30,000 MW cut-off ultrafiltration membrane. Formaldehyde and lysine solutions were added to a final concentration of 0.3 and 0.9% w/v respectively and toxoiding was allowed to proceed for 2 weeks at 37 °C. The resultant toxoid was purified by ammonium sulphate precipitation.

Tetanus toxoid was adsorbed to the aluminium salt adjuvant (aluminium hydroxide or aluminium phosphate) by slow addition of the antigen to the suspension of aluminium adjuvant whilst continuously stirring. The stirring was continued overnight. The aluminium hydroxide gel was sourced as "Alhydrogel" from Superfos, Denmark. The aluminium phosphate gel was prepared by back titration of a solution of aluminium chloride with tri-sodium phosphate. When desired, stabiliser was dissolved in water to a concentration of 50% (w/v) then added to the adsorbed tetanus toxoid to give the required final concentration as stated in Table 1.

The aqueous suspension of aluminium salt-adsorbed tetanus toxoid was spray-dried in a Drytec Compact Laboratory Spray Dryer equipped with a 40/100/120 concentric-type nozzle at an atomising pressure of 80 psi and an outlet temperature of 60 °C. The resultant microspheres had a size range around 3 μm in diameter and were collected as a free-flowing powder.

EXAMPLE 2

Preparation of an immediate-release diphtheria vaccine.

Cornyebacterium diphtheria was cultured in a medium incorporating casein hydrolysate modified to have a total nitrogen content of 0.2% (w/v) and containing 1.5% (w/v) maltose.

Seed was grown as a 24 hour surface culture in tubes then inoculated into 250 ml volumes in 500 ml Erlenmeyer flasks which were incubated at 35 °C for 3 days on a table rotating at 200 rpm.

Toxin was clarified by filtration to remove bacteria, concentrated to 1% the original volume by ultrafiltration (50,000 MW cut-off) then washed at that volume with half the original volume of PBS. Final purification was on a Sephadex G-100 column, to a purity of 2200 Lf/mg protein nitrogen. The procedure is described in detail by Cox (1975). Formaldehyde and lysine solutions were added to a final concentration of 0.3% and 0.9% (w/v) respectively and toxoiding was allowed to proceed for 4 weeks at 37 °C.

Diphtheria toxoid was absorbed to the aluminium salt adjuvant as described previously for tetanus toxoid, and the aqueous suspension of aluminium salt-absorbed diphtheria toxoid was spray-dried as described previously (Example 1).

EXAMPLE 3

Preparation of an immediate-release botulinum C & D vaccine.

Clostridium botulinum strains C and D were grown in a cellophane-sac apparatus modified from Sterne (1958). Growth medium external to the sac was a modified corn steep medium which was allowed to equilibrate with PBS within the dialysis sac. Seed cultures of *C.botulinum* were inoculated into the PBS and incubated at 37 °C for 18 days under anaerobic conditions. The contents of the dialysis sac were then harvested, cells removed by centrifugation and formaldehyde to a final concentration of 0.5% (w/v) added. Toxoiding was allowed to occur at 37 °C until complete (7-14 days) then potency was determined as described in the British Pharmacopoeia-Veterinary (1985).

Botulinum toxoids type C and D were mixed with Quil A (Superfos) and spray-dried as described previously for tetanus toxoid (Example 1).

EXAMPLE 4

Preparation of Bordetella pertussis derived PTD immediate-release vaccines.

Cultures of *Bordetella pertussis* were grown in shake flasks in a modified Stainer and Sholte medium (Stainer & Sholte, 1970) containing 1 mg/ml 2,6 di-methyl β cyclodextrin. The flasks were incubated at 37 °C with gentle agitation at 180 rpm for 42 hrs when a cell density of around 2.0 x 10^{10} organisms/ml was achieved.

Pertussis toxin (PTX) was purified from the culture supernatant after clarification by filtration. PTX was bound specifically to asialofetuin by affinity chromatography essentially as described by Sekura *et al.* (1985), washed, then eluted with 50mM Tris/4M urea buffer, pH 9.0.

PTX was toxoided at pH 9.6 in the presence of 2.5 mM glutaraldehyde for 48 hrs at 4 °C when reaction was terminated by addition of 9 mM lysine. The method was essentially as described in Australian Patent Specification No. 601415 (71581/87). The resultant pertussis toxoid (PTD) was adsorbed to the aluminium salt adjuvant and spray-dried as described previously.

EXAMPLE 5

Preparation of Delayed-Release Tetanus Vaccine

A. Emulsion Procedure.

50:50 and 85:15 copolymers of polylactide and polyglycolide (PLG) and the homopolymer of polylactic acid (PLA) were obtained from Birmingham Polymers Ltd., Birmingham, Alabama, USA. The copolymers were solubilised to 10% w/v dissolution in either chloroform or a mix of 5 parts of trichloroethylene and 3 parts of 1,1,2-trichloroethane. For each of these polymer solutions, an emulsion was produced as follows:

(a) to 93 parts of polymer solution were added 1 part of soya lecithin and 6 parts of an aqueous suspension of aluminium salt-adsorbed tetanus toxoid, or

(b) to 88 parts of polymer solution were added 1 part of a 1:5 mixture of Tween 80 and Span 85 and 11 parts of an aqueous suspension of aluminium salt-adsorbed tetanus toxoid.

The production of the aqueous suspension of aluminium salt-adsorbed tetanus toxoid is described in Example 1 above. The mixture was vigorously agitated using either an ultrasonic probe or a high-speed blender (e.g. a Silverson blender) to produce a stable water-in-oil emulsion with a milk-like consistency and appearance. This emulsion was spray-dried using a Drytec Compact Laboratory Spray Dryer equipped with a 40/100/120 concentric-type nozzle at an atomising pressure of 30 psi and an outlet temperature of 35 °C. The resultant microspheres had a size range around 30 µm in diameter and were collected as a free-flowing powder. Traces of remaining organic solvent were removed by vacuum evaporation. A number of preparations were made to permit consideration of the following variables:

(a) choice of polymer - 50:50 PLG
85:15 PLG
PLA

(b) choice of adjuvant - aluminium hydroxide
- aluminium phosphate

(c) choice of stabiliser - 0, 0.5 and 5.0% trehalose.

B. **Suspension Procedure:**

Polymer solutions were prepared as described in Section A above, then microspheres of particulate immediate-release aluminium salt-adsorbed tetanus toxoid, prepared as described in Example 1, were added to a final 1% w/v suspension. The mixture was agitated sufficiently to maintain an even suspension and spray-dried as described in Section A above to a particle size around 30 µm. In some experiments, tetanus toxoid, spray-dried to small microspheres but in the

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9. Sterne, M. The growth of *Brucella abortus* strain 19 in aerated dialysed medium. *J. Gen. Micro.* 18, 747, 1958.

10. British Pharmacopoeia-Veterinary (1985). Her Majesty's Stationery Office, London.

11. Stainer, D. and Sholte, M. A simple chemically defined medium for the production of Phase I *Bordetella pertussis* *J. Gen. Micro.* 63, 211, 1970.

12. Sekura, R.D., Zhang, Y.I. and Quentin-Millet, M-J.B. Pertussis toxin : Structural elements involved in the interaction with cells. Pertussis toxin. Ed. Sekura *et al.*, Academic Press, p.45, 1985.

CLAIMS:

1. An immediate-release vaccine preparation in stable particulate form, comprising an immunogen adsorbed to an aluminium salt adjuvant.

2. A vaccine preparation of claim 1, wherein said aluminium salt adjuvant is aluminium hydroxide or aluminium phosphate.

3. A vaccine preparation of claim 1 further comprising a protein stabiliser.

4. A vaccine preparation of claim 3, wherein said stabiliser is a sugar or sugar derivative.

5. A vaccine preparation of claim 4 wherein said stabiliser is selected from the group consisting of trehalose, lactose, dextrose and glucosamine.

6. A vaccine preparation of claim 1, wherein said particulate form is a free flowing powder.

7. A method for the production of a vaccine preparation of claim 1, which comprises the steps of forming an aqueous suspension of aluminium salt-adsorbed immunogen, and subsequently spray-drying said suspension.

8. A controlled- or delayed-release vaccine preparation in stable particulate form, said particles being microspherical particles comprising a continuous matrix of biodegradable polymer containing discrete, immunogen-containing regions.

9. A vaccine preparation of claim 8, wherein said immunogen-containing regions also comprise an adjuvant.

10. A vaccine preparation of claim 8, wherein said immunogen-containing regions contain particles comprising an immunogen adsorbed to an aluminium salt adjuvant.

11. A vaccine preparation of claim 8, wherein said biodegradable polymer is selected from the group consisting of polylactic acid, polyglycolic acid, and copolymers thereof.

12. A method for the production of a vaccine preparation of claim 8, which comprises the steps of forming an emulsion of an aqueous suspension comprising immunogen and optionally an adjuvant in a continuous organic phase having biodegradable polymer dissolved therein, and subsequently spray-drying the water-in-oil emulsion to form microspherical particles.

13. A method of claim 12, wherein said emulsion includes an emulsifier.

14. A method for the production of a vaccine preparation of claim 8, which comprises the steps of forming a suspension of a particulate immunogen-containing material and optionally an adjuvant in a continuous organic phase having biodegradable polymer dissolved therein, and subsequently spray-drying the suspension to form microspherical particles.

15. A method of claim 14, wherein the particulate immunogen-containing material comprises an immunogen adsorbed to an aluminium salt adjuvant.

16. A vaccine composition comprising at least one immediate-release vaccine preparation of any of claims 1 to 6, together with a pharmaceutically or veterinarily acceptable carrier or diluent.

17. A vaccine composition comprising at least one controlled or delayed-release vaccine preparation of any of claims 8 to 11, together with a pharmaceutically or veterinarily acceptable carrier or diluent.

18. A vaccine composition of claim 17 further comprising at least one immediate-release vaccine preparation in stable particulate form.

19. A vaccine composition of claim 18, wherein said immediate-release vaccine preparation comprises an immunogen adsorbed to an aluminium salt adjuvant.

20. A vaccine composition of any of claims 16 to 19 in a form suitable for parenteral administration.

21. A vaccine composition of any of claims 16 to 20, wherein said carrier is a solid carrier and said vaccine composition is in the form of a solid pellet or implant.

22. A method of vaccinating a human or other animal patient, which comprises administration to the patient of a vaccine composition of any of claims 16 to 21.

AMENDED CLAIMS

[received by the International Bureau on 26 April 1994 (26.04.94); original claims 1 and 8 amended; remaining claims unchanged (1 page)]

1. (Amended) An immediate-release vaccine preparation in stable particulate form prepared by spray-drying, comprising an immunogen adsorbed to an aluminium salt adjuvant.

2. A vaccine preparation of claim 1, wherein said aluminium salt adjuvant is aluminium hydroxide or aluminium phosphate.

3. A vaccine preparation of claim 1 further comprising a protein stabiliser.

4. A vaccine preparation of claim 3, wherein said stabiliser is a sugar or sugar derivative.

5. A vaccine preparation of claim 4 wherein said stabiliser is selected from the group consisting of trehalose, lactose, dextrose and glucosamine.

6. A vaccine preparation of claim 1, wherein said particulate form is a free flowing powder.

7. A method for the production of a vaccine preparation of claim 1, which comprises the steps of forming an aqueous suspension of aluminium salt-adsorbed immunogen, and subsequently spray-drying said suspension.

8. (Amended) A controlled- or delayed-release vaccine preparation in stable particulate form, said particles being microspherical particles prepared by spray-drying comprising a continuous matrix of biodegradable polymer containing discrete, immunogen-containing regions.

9. A vaccine preparation of claim 8, wherein said immunogen-containing regions also comprise an adjuvant.

10. A vaccine preparation of claim 8, wherein said immunogen-containing regions contain particles comprising an immunogen adsorbed to an aluminium salt adjuvant.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 93/00677

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl.[5] A61K 39/39, 39/08, 39/10, 39/05, 47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC[5] A61k 39/39
CHEMICAL ABSTRACTS

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
DERWENT FILE WPAT; A61K/IC, ALUM:(S) ADJUV:, TETAN:, DIPHTHER:, BOTULIN:, BORDATELLA() PERTUSSIS; FILE CASM
MEDLINE DATABASE.
BIOSIS; CODED SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	AU,A, 41876/89 (THE AUSTRALIAN NATIONAL UNIVERSITY) 23 March 1990 (23.03.90) see pages 6-8, claims 1-5 in particular	1-2, 6, 8-9
X	AU,A, 29557/89 (MICROGENESYS, INC) 3 August 1989 (03.08.89) see Example 9 in particular	1-2, 6, 16, 20-22
P,X	US 5242686 (Chu et al.) 7 September 1993 (07.09.93) Priority Date 7 November 1990 (07.11.90) see column 7, line 7-column 8, line 14	1-2, 6-22

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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10 March 1994 (10.03.94)	18 March 1994 (18.03.94)
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. 06 2853929	Authorized officer H. Flame H FLAME Telephone No. (06) 2832253

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 93/00677

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
X	CH,A5, 645270 (SCHWEIZERISCHES SERUMUND IMPFINSTITUT, BERN) 28 September 1984 (28.09.84) see entire document	1-2

INTERNATIONAL SEARCH REPORT

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Box I **Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)**

This international search report has not established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

I Claims 1-7, 10, 15-22: Vaccines characterized by an aluminium salt adjuvant, methods of preparation.

II Claims 8-9, 11-14: Vaccines characterized by biodegradable polymer microspherical particles.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically

claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family mem.

International application No.
PCT/AU 93/00677

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member							
AU	41876/89	EP	431023	JP	4501105	WO	9001949	
AU	29557/89	AU	25206/92	BR	8900515	EP	327180	
		IL	89118	JP	2203793	ZA	8900862	
US	5242686							
CH	645270							

END OF ANNEX